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Original Article

Application of contrast-enhanced ultrasound in evaluating plaque vulnerability after extracranial carotid artery stenting and analysis of risk factors for unstable plaques

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Abstract: *Objectives:* To evaluate the efficiency of contrast-enhanced ultrasound (CEUS) in identifying plaque vulnerability after extracranial carotid artery stenting and the relevant risk factors for unstable plaques after CAS. *Methods:* CEUS was performed on 47 plaque lesions in 40 patients. Plaque enhancements were quantitatively evaluated and the lesions were classified as stable (grades 0-1) and vulnerable (grade 2). The correlations between the vulnerability of the below-stent plaques and clinical characteristics as well as blood biochemical data were analyzed. *Results:* CEUS revealed four (8.5%) grade 0 lesions, 15 (31.9%) grade 1 lesions, and 28 (59.6%) grade 2 lesions. 43 lesions developed newly formed blood vessels and could be graded based on CEUS measurement, indicating that the plaques below the stents were still vulnerable. Ultimately, 14 stable plaques and 26 vulnerable plaques were subject to multivariate logistic analysis, and the results showed that only serum triglyceride level was significantly correlated with the presence of vulnerable plaque lesions after stenting (P=0.044, 95% confidence interval (CI)=1.069-121.361), suggesting that triglyceride level is an independent risk factor for the presence of vulnerable plaque after stenting. *Conclusions:* CEUS showed that, after extracranial carotid artery stenting, plaque lesions were still vulnerable and the vulnerability could be graded. The combination of clinical characteristics and biochemical data can be used for more accurate evaluation and prediction of the occurrence of vulnerable below-stent plaques.

Keywords: Post extracranial carotid artery stenting, ultrasound imaging, vulnerable plaque, medical history, biochemical indicators, risk factors

Introduction

Atherosclerotic plaque resulting from carotid stenosis has been shown to be the primary culprit in ischemic diseases [1]. In addition, carotid atherosclerotic plaque lesions that cause severe stenosis are often highly vulnerable [2, 3]. Rupture, bleeding, and dislodgement of vulnerable plaques are more dangerous than the stroke that results from significant stenosis [4]. Currently, the main treatment strategies for extracranial carotid artery (ECA) stenosis are carotid endarterectomy (CEA) and carotid artery stenting (CAS). Nowadays, CAS is becoming a predominate surgical method for carotid artery stenosis, which not only alleviates the stenosis and improves intracranial blood circulation, but also structurally stabilizes the plaque lesions that cause the stenosis. However, the lesions are still physically present after stenting, and no study has evaluated their likelihood of becoming unstable. The association between vulnerability of postoperative stent plaques and in-stent restenosis (ISR) has not been reported. Ultrasound imaging can show intralesional neovascularization and thus indirectly evaluate plaque vulnerability [5], but its application in determining plaque vulnerability after CAS has

not been reported. In the present study, we performed CEUS in 40 patients with extracranial carotid artery stenosis who underwent CAS. Medical histories of those patients were recorded. Relevant biochemical blood tests were performed in the fasting state on the morning of the imaging examination. We determined correlations between traditional cardiovascular risk factors and post-CAS plaque vulnerability to provide a theoretical basis for preventing post-CAS restenosis.

Materials and methods

Study design and patients

40 patients (36 males and 4 females; mean age 64.83 ± 9.38 years [range, 40-78 years]) admitted to our department and underwent CAS for ECA stenosis were retrospectively recruited in this study between September 2014 and May 2015. All patients had regular follow-up examinations after CAS, and one staff member of this study was dedicated to obtain medical records and provide guidance on medication regimens (aspirin, statins, and other medications to control risk factors). Follow-up examinations included blood pressure measurement, non-enhanced ultrasound examination, and venous blood sampling to determine blood glucose and lipid levels, kidney function, homocysteine, and high-sensitivity C-reactive protein (HsCRP).

Ultrasound equipment and contrast agent

Ultrasound imaging was performed at a LOGIQ™ E9 system (GE Healthcare, Little Chalfont, UK), using a 9L, 7- to 12-MHz probe and image analysis software. Real-time ultrasound images were obtained under Contrast General Mode and the mechanical parameter was set at 0.13, with gain at 15. The focal position was approximately 3-5 cm. The same equipment parameter settings were used for all patients.

SonoVue® (Bracco Imaging S.p.A., Ceriano, Italy) ultrasound contrast medium (25 mg) was suspended in 5 mL of normal saline. A 2-mL bolus was administered intravenously in the left median cubital vein, followed by a 5-mL normal-saline flush at the same speed.

Body positioning and imaging technique

The potential risks during the procedure, including allergic reaction to contrast medium, were explained to the patients, and written informed consents were obtained. For each procedure, the patient was placed in a supine position with the head turned for full exposure of the neck on the examined side. A two-dimensional ultrasound was first performed, longitudinally from proximal to distal and transversely, to image the common carotid artery and bifurcation, internal and external carotid arteries, vertebral artery, and subclavian artery. Internal diameter, flow velocity, intima-media thickness, and plaque position, size, and echo signals were recorded to determine the optimal observation position for each plaque lesion. Ultrasound imaging was then performed with the preset parameters. First, the lesions below the stent were examined. Contrast injection and the built-in imaging timer were started simultaneously. The probe was fixed during the examination and the observation lasted for 80 s, with automatic collection of the sequences. Mobile, high-frequency echo signals within the plaque suggested enhancement. The grading system established by Coli et al [6] was used to stratify the lesion enhancement (0, no enhancement; 1, slight enhancement; and 2, extensive enhancement). If examination of the contralateral side was needed, the same procedure was performed 15 min later to allow complete clearance of the contrast medium. Two experienced senior physicians who specialized in ultrasound imaging evaluated the images for degree of plaque enhancement and assigned a grade to each lesion.

Diagnostic criteria

Inclusion criteria were (1) underwent CAS, (2) follow-up digital subtraction angiography indicating postoperative stenosis of <30%; (3) all plaque lesions located at the distal 1.5 mm of the common carotid artery, carotid bulb, or within 1.0 mm of the origination segment of the internal carotid artery; and (4) lesions below the stent, with low or mixed echo, and ≥2.0 mm thick.

Exclusion criteria were (1) complete stent obstruction and ultrasound imaging unnecessary; (2) carotid plaque present without stent deployment; (3) plaque thickness <2.0 mm, with calcification and apparent echo signals; (4) cardiac dysfunction, impaired liver or kidney function; and endocrine, metabolic, or connective tissue lesions; (5) allergy, particularly to the contrast medium.

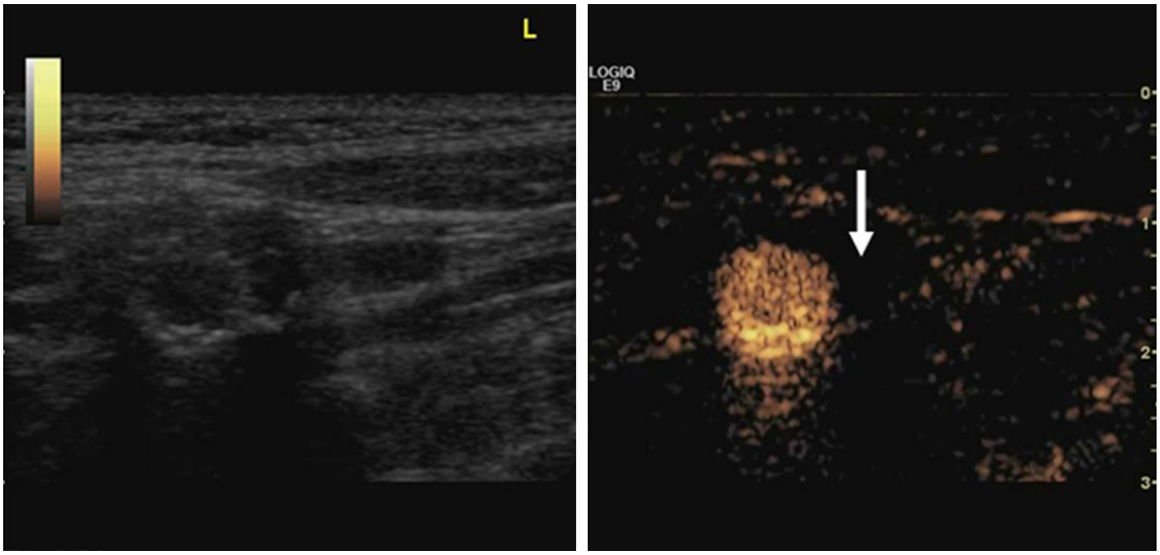

Figure 1. Ultrasound of the left carotid sinus in the transverse plane revealing a low-echo plaque lesion below the stent and the absence of contrast agent in grade 0 plaque (thick arrow).

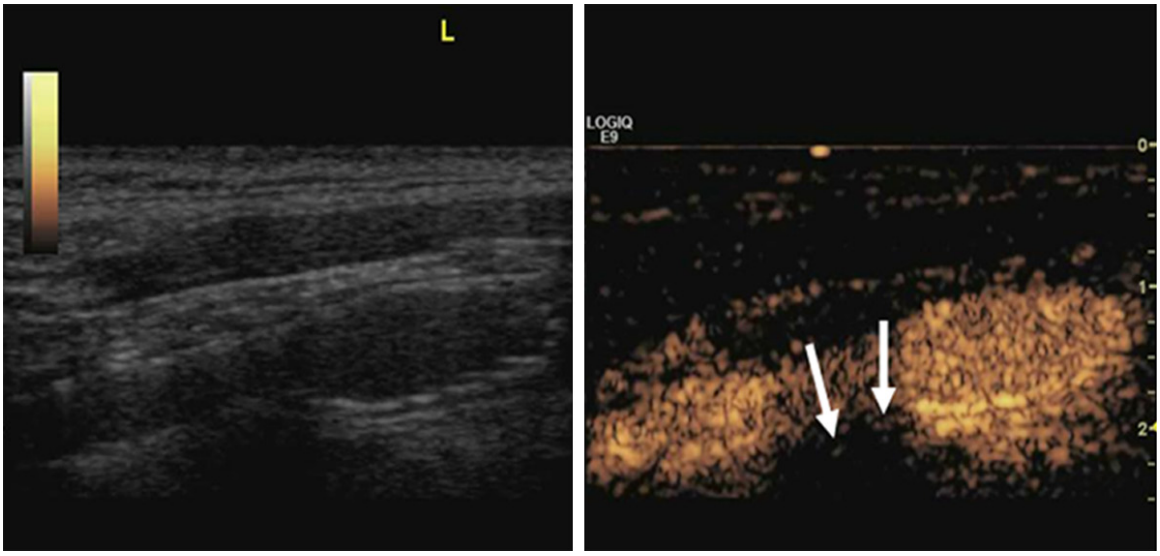

Figure 2. Ultrasound of the left carotid sinus posterior wall revealing low-echo plaque below the stent and the spotty appearance of contrast agent in grade 1 plaque.

Diagnostic criteria were as follows: diabetes mellitus: fasting blood sugar ≥7.0 mmol/L, or random blood sugar ≥11.1 mmol/L, glycosylated hemoglobin (HbAlc) ≥6.5%; high blood pressure: systolic pressure ≥140 mmHg or diastolic pressure ≥90 mmHg; abnormal lipids: triglycerides (TG) >1.6 mmol/L; high-density lipoprotein cholesterol (HDL-C) <1.96 mmol/L; low-density lipoprotein cholesterol (LDL-C) >3.10 mmol/L; total cholesterol (TC) >5.50 mmol/L; homocysteine (Hcy): male, 0-15 μmol/L, female, 0-20 μmol/L; high-sensitivity C-reactive protein, 2.1-3 mg/L; kidney dysfunction, estimated glomerular filtration rate (eGFR), 100 ± 20 mL/min; smoker, >10 cigarettes per day for over 5 years; positive alcohol consumption, >100 g liquor per day, or >1.5 bottles of beer per day, for more than 5 years.

Statistical analysis

SAS® software, Version 9.3 (SAS Institute Inc., Cary, NC, USA) was used for data analysis.

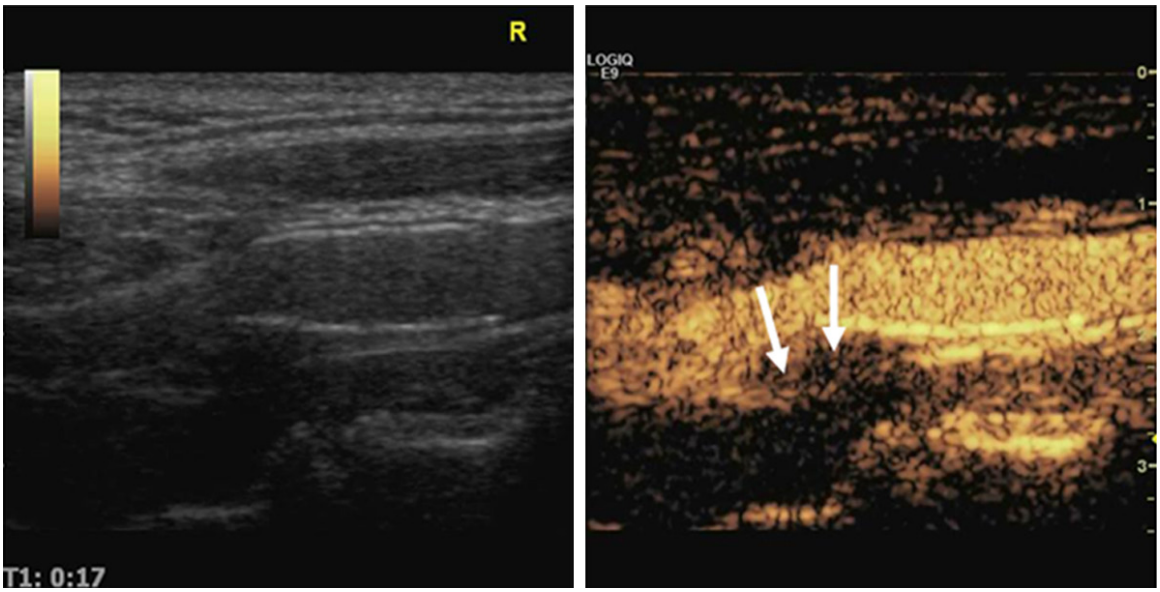

Figure 3. Posterior ultrasound of the right carotid sinus posterior wall revealing the low-echo plaque below the stent and the presence of a large amount of contrast agent diffusely in grade 2 plaque.

Quantitative data were expressed as mean ± standard deviation, and categorical data were described using n (%). Between-group comparison of quantitative data was performed using Student's *t* test and categorical data were analyzed using chi-square test, respectively. Univariate analysis was performed using logistic regression and multivariate analysis using stepwise logistic regression. Statistical significance level was $\alpha<0.05$.

Results

Forty patients, two of whom had bilateral CAS, were included in the analysis (42 stented carotid arteries with 47 below-stent plaque lesions). Plaque lesions were located on both the anterior and posterior walls in five cases. Grading of CEUS images was as follows: grade 0, four soft plaques (8.5%) (**Figure 1**); grade 1, 15 plaques (13 soft and three mixed; 31.9%) (**Figure 2**); and grade 2, 28 plaques (20 soft and 8 mixed; 59.6%) (**Figure 3**). Forty-three plaques had visible new blood micro-vessels that could be semi-quantified, indicating that the plaques below the stents were still vulnerable. In addition, abnormal intimal thickening (0.7-1.2 mm) developed within the stents in five cases (12.5%) during postoperative 3, 6, 12, 18, and 24 months. In one case (2.5%), newly formed plaque lesions were seen within the stent 6 months postoperatively; in another case, severe stenosis developed within the stent during the first year after surgery. Imaging results of those patients showed moderate thickening of the below-stent lesions, suggesting relatively low stability.

Comparison of risk factors between patients with stable and vulnerable plaque lesions

Lesions were graded by degree of enhancement on postoperative ultrasound imaging. Patients were divided into stable plaque (grades 0 and 1, stable group; n=14) and vulnerable plaque (grade 2, vulnerable group, n=26) groups. If plaque lesions were presented on both sides of the stent or on both the anterior and posterior stent walls and the corresponding enhancements differed, lesion was assigned the higher grade. If the enhancements were identical, only one was recorded. Analysis of relevant risk factors demonstrated the majority of factors were comparable in the two groups except for TG and Hcy levels (**Table 1**).

Analysis of risk factors for post-CAS vulnerable plaque lesions

Univariate logistic regression analysis was performed for 18 variables to compare stable-with vulnerable-plaque groups. Coronary heart disease was rare in the study and was therefore was removed from the analysis. TG was the

Table 1. Comparison of risk factors between stable and vulnerable groups

	Stable plaques (grades 0-1)	Vulnerable plaques Grade 2	*P* value
Number of cases	n=14	n=26	
Sex (Male)	13 (92.86%)	23 (63.89%)	0.3983
Age (year)	65.14 (7.93)	64.65 (10.22)	0.8774
Smoking status	8 (57.14%)	18 (69.23%)	0.4446
Drinking status	7 (50.00%)	6 (23.08%)	0.0657
High blood pressure (mmHg)	12 (85.71%)	21 (80.77%)	0.3211
Diabetes	6 (42.86%)	10 (38.46%)	0.2538
Coronary heart diseases	0 (0.00%)	5 (19.23%)	0.1000
TG	1.02 (0.36)	1.44 (0.64)	0.0117
Total cholesteral	3.42 (0.82)	3.38 (0.94)	0.9080
HDL	1.16 (0.32)	1.12 (0.27)	0.6981
LDL	1.80 (0.65)	1.74 (0.77)	0.8248
GFR (ml/min)	74.71 (27.22)	70.77 (46.02)	0.7709
GLU (mmol/L)	5.17 (0.78)	6.06 (2.15)	0.0644
Hcy (μmol/L)	17.79 (7.76)	28.67 (24.11)	0.0464
HsCRP (mg/L)	0.93 (0.87)	1.32 (2.34)	0.5116
Plaque echo (mixed plaques)	1 (7.14%)	7 (26.92%)	0.1197
Plaque thickness (mm)	2.81 (0.83)	2.57 (0.64)	0.3051
Duration after surgery (month)	20.7 (19.71)	17.17 (13.24)	0.4975

Data presented as mean (standard deviation) or n (%). eGFR: estimated glomerular filtration rate; GLU: glucose; Hcy: homocysteine; HDL: high-density lipoprotein; HsCRP: high-sensitivity C-reactive protein; LDL: low-density lipoprotein; TG: triglyceride.

only risk factor significantly associated with the presence of vulnerable plaque, with a greater than 5-fold increase of risk for developing vulnerable plaque by each mmol/L increase in TG (odds ratio [OR]=5.376; *P*=0.0467, 95% confidence interval [CI]=1.025-28.195) (**Table 2**). 18 variables were then screened to remove those that were not significant, and stepwise multivariable logistic regression was then performed. The corrected OR value still showed that TG was a significant risk factor for post-CAS vulnerable plaque lesions (*P*=0.0438, 95% CI=1.069-121.361), with an odds ratio demonstrating a more than 11-fold increased risk for the development of vulnerable plaque for each mmol/L increase in TG (OR=11.392) (**Table 3**).

Discussion

The pathologic characteristics of vulnerable plaque lesions resulting from atherosclerotic disease of the carotid artery are a large lipid core with thin fibrous cap as well as active inflammation and angiogenesis, which are associated with increased risk of plaque rupture [7]. The combination of inflammation and angiogenesis are essential to promote the spread of atherosclerosis and increase the risk of vulnerable plaque, eventually leading to ischemia. The development of various imaging technologies in recent years have provided a deeper, multiple-perspective understanding of vulnerable plaque that may cause stroke [8-11], but these technologies have some limitations. Ultrasound imaging not only allows visualization of new and feeder blood vessels within the lesions, with the similar relative density of microvessels within the plaque tissues [6, 12], but it is also widely considered a convenient and reliable method for assessment of plaque stability [13]. In recent years, CAS, an effective, minimally invasive treatment method for extracranial carotid artery stenosis that can rapidly open stenotic blood vessels and restore intracranial blood supply [14], has challenged and gradually replaced CEA, which has long been considered the gold standard. However, plaque lesions are still present after CAS, and their stability can change, and acute stimulation by a stent as well as risk factors that persist after the procedure can also affect the postoperative stability of a lesion and lead to postoperative restenosis. For these reasons, we began to investigate the use of CEUS technology to evaluate new blood vessels within plaque lesions after CAS.

In the present study, signal enhancement was observed in all but four below-stent lesions (91.5%). Of these, 15 were grade 1 and 28 were grade 2, indicating that new blood vessels could still form within the below-stent lesions and lead to progression to a higher grade and persistently vulnerable plaque. These results suggest that the external force of the stents caused the plaques to shrink; however, the number of newly formed blood vessels was not

Table 2. Univariate analysis of risk factors for vulnerable plaques after CAS

Risk factors	OR value (95% CI)	*P* value
Sex (Female)	1.695 (0.160~18.00)	0.6616
Age (year)	0.994 (0.927~1.067)	0.8735
Smoking	1.688 (0.439~6.489)	0.4464
Drinking	0.300 (0.075~1.203)	0.0894
High blood pressure (mmHg)	0.700 (0.117~4.179)	0.6956
Diabetes	0.833 (0.222~3.122)	0.7867
TG	5.376 (1.025~28.195)	0.0467
Total cholesterol	0.956 (0.457~2.001)	0.9050
HDL	0.625 (0.062~6.266)	0.6897
LDL	0.900 (0.366~2.217)	0.8194
eGFR (ml/min)	0.998 (0.981~1.014)	0.7641
GLU (mmol/L)	1.609 (0.857~3.021)	0.1387
Hcy (umol/L)	1.048 (0.983~1.116)	0.1484
HsCRP (mg/L)	1.134 (0.679~1.896)	0.6307
Plaque echo (mixed plaques)	4.787 (0.525~43.65)	0.1650
Plaque thickness (mm)	0.617 (0.248~1.537)	0.2996
Duration after surgery (month)	0.985 (0.945~1.027)	0.4892

eGFR: estimated glomerular filtration rate; GLU: glucose; Hcy: homocysteine; HDL: high-density lipoprotein; HsCRP: high-sensitivity C-reactive protein; LDL: low-density lipoprotein; TG: triglyceride.

Table 3. Multivariate logistic analysis of risk factors for the development of vulnerable plaque lesions after CAS

Risk factors	Corrected OR value (95% CI)	*P* value
TG	11.392 (1.069~121.361)	0.0438
HDL-C	82.545 (0.722~>999.999)	0.0679

HDL-C: high-density-lipoprotein cholesterol; OR: odds ratio; TG: triglyceride.

reduced. Regular ultrasound evaluates only plaque morphology, and the strong echoes from the stents can interfere with the echoes within the plaques. Thus, two-dimensional grayscale ultrasonography may not provide an accurate evaluation of plaque stability. CEUS provides information about newly formed blood vessels within the lesions, which is more accurate in clinical practice.

In the present study, univariate and multivariate regression demonstrated TG to be the only significant predictor of vulnerable plaque after CAS. Risk of vulnerable plaque increased 11.392-fold for each mmol/L increase in TG; i.e., as TG level increased, the enhancement grade of below-stent lesions increased, and the plaques were more vulnerable. In a study by Grønholdt et al [15], an increased concentration of TG lipid protein predicted low-echo carotid artery plaque lesions, particularly vulnerable lesions, indicating that those lesions with increased TG and low echoes were vulnerable, and increased TG levels were significantly correlated with vulnerable plaque lesions of the carotid artery. Therefore, because suboptimal risk-factor control after surgery can affect the severity of the plaques below the stents, early identification of risk factors is important for the prevention and treatment of vulnerable plaque.

Moreover, because inflammatory response is a primary feature of vulnerable plaque, blood inflammatory factors such as Hcy, CRP, matrix metalloproteinases, and interleukin as indirect indicators of plaque vulnerability have also been investigated. Xu et al [16] showed that serum Hcyw as a risk factor for vulnerable plaque and could be used for indirect determination of plaque vulnerability via intravascular ultrasound. Xiaoni et al [17] performed CEUS on 72 plaques in 48 patients. The lesions were classified into 3 groups by degrees of enhancement, and serum CRP level was identified as a risk factor. Results showed that the enhancement of the lesions gradually increased with serum CRP levels, and both between-group and within-group comparisons revealed statistical significance ($P<0.05$). Correlation analysis showed that plaque enhancement was significantly positively correlated with serum CRP levels. These results suggested that the combination of ultrasound evaluation of the newly formed micro blood vessels within the plaques and serum CRP value could accurately predict plaque vulnerability. However, in the present study, Hcy differed significantly between groups ($P=0.0464$), but was not a significant factor in univariate or multivariate analysis. HsCRP was also not a significant predictor of plaque vulnerability. One possible explanation is the retrospective nature of the present study, which could have led to selection bias, and its small sample size. We plan to have future investigations to provide further evidence for predicting the vulnerability of below-stent plaque lesions.

Although CAS relieves stenosis, the two major complications, ISR and stroke, are challenging for neuro-interventionists and neurosurgeons. Recent studies have revealed a gradual increase in the incidence of ISR, which has been shown to be caused mainly by intimal thickening of the newly formed blood vessels. Some researchers compared the different stages of the pathological processes of atherosclerotic plaque and ISR, and showed that both diseases have nearly identical pathological changes [18-20]. The difference is that the former is a chronic process that develops over many years, whereas the latter is an acute process that begins with mechanical stimulation by the stents and then becomes stable [21]. In clinical practice, CEUS has been used successfully to identify vulnerable plaque lesions and prevent stokes; however, the use of such technology to investigate the mechanism of postoperative ISR and subsequent intervention has not been reported.

In the present study, intimal thickening developed in five cases, the plaque lesion re-formed within the stent in one case, and severe restenosis within the stent developed in one case. Most (71.4%) occurred within the first year after surgery, consistent with the findings of Wasser et al [22]. The imaging results of these patients also revealed relatively high enhancement within the plaque lesions, which might be critical in ISR development. We speculated that stent placement physically stimulated and damaged the endothelial cells, which led to endothelial dysfunction, and the initiation of inflammatory response, intimal hyperplasia and repair, feeder blood vessel formation, new blood vessel formation, and proliferation/migration of the smooth muscle cells crossed the stent interspace and resided into the stent [19, 23]. Various risk factors simultaneously promoted over-repair of the endothelium, but the involvement of inflammatory responses and feeder blood vessels was critical [24]. In an animal model of abdominal aortic atherosclerosis in the presence of balloon dilation using rabbits, conducted by Ciannareli et al [25], CEUS results showed that more new blood vessels were formed within the plaque lesions following balloon damage when compared with the control group. The balloon-damaged group, but not the control group, had enhanced images and more distinct inflammatory infiltration. Similar studies have shown that CEUS demonstrated a linear correlation between the adventitial blood vessel density and histologic nutrient blood vessel density in arteriosclerosis resulting from balloon damage and could predict the formation of new blood vessels [26]. Similarly, we showed that the relatively high enhancement within below-stent plaque lesions observed in seven cases and might have been caused by a series changes resulting from mechanical damage by the stent. However, while the number of cases was low and the correlation between vulnerability of below-stent plaque and ISR could not be definitively determined, we will develop new ideas and methods for a future study of the mechanism of ISR.

The present study had some limitations. First, the sample size was small. Second, the study was retrospective in nature and did not compare vulnerable plaque lesions before and after stenting. The third one, this first application of ultrasound imaging to evaluate below-stent plaque lesions had a number of interfering factors, which could have decreased the accuracy of our results.

In summary, we combined ultrasound imaging with medical history and biochemical tests for a more accurate evaluation and prediction of plaque vulnerability below the stent. However, the reliability of these preliminary results needs further confirmation in a larger sample.

Disclosure of conflict of interest

None.

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